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Saxicolous species of *Claudopus* (*Agaricales*, *Entolomataceae*) from Australia

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Abstract — *Claudopus rupestris*, *C. viscosus,* and *C. minutoincanus* are described as new to science. All three species produce abundant, white basidiomata on the underside of granitic rocks and have a pileus that is sticky to the touch. *Claudopus rupestris* has unusual basidiospores, some of which are 4-angled. *Claudopus viscosus* and *C. minutoincanus* possess subcapitulate to capitulate pileocystidia and are morphologically similar to *Entoloma jahnii* of Europe.

Key words — *Basidiomycota*, mtSSU, LSU, RPB2, novel species

Introduction

The genus *Claudopus* Gillet is considered a subgenus within the genus *Entoloma* (Fr.) P. Kumm. by some researchers (Noordeloos 1981, 1992, 2004, Manimohan et al. 2006). Others consider it an independent genus (Dennis 1970, Horak 1980, 2008, Largent 1994, Pegler 1983), a concept favored in this report. Because of the ease with which these species can be identified macroscopically, those who do not recognize *Claudopus* as either an autonomous genus or as a subgenus within *Entoloma* still place species into a key group based on the distinct stature of the basidiomata (Hesler 1967, Knudsen & Vesterholt 2008).

Claudopus can be distinguished from pleurotoid species of *Clitopilus* (Fr. ex Rabenh.) P. Kumm. or *Rhodocybe* Maire by their basidiospores, which are angular in all views. Basidiospores are angular only in polar view in *Clitopilus* and angular-pustulate in all views in *Rhodocybe*.

Claudopus species, which are easily recognized by their pleurotoid stature, possess a centrally attached stipe that typically becomes eccentric, lateral, or

disappears completely as the pileus expands and a pileus that is either matted-fibrillose or has a fibrillose covering.

Although *Claudopus* species are cosmopolitan, basidiomata are often overlooked by researchers because of their small size or unusual habitats. As a consequence, researchers typically describe only one or two species per publication (e.g. Horak 2008, Manimohan et al. 2006, Pegler 1977, 1983) and often include none in floristic studies (Romagnesi & Gilles 1979). Nonetheless, Noordeloos (2004) reported ten species from Europe, and Horak (1980) included five from Indomalaya and Australasia.

Only two species, *Claudopus byssisedus* (Pers.) Gillett and *C. depluens* (Batsch) Gillet, have been reported from Australia (May & Wood 1997). Although authentic Australian material does exist for *C. byssisedus*, all Australian *C. depluens* material has been re-identified as a species of *Crepidotus* (Fr.) Staude (Horak 1980, May & Wood 1997).

We report here three *Claudopus* species that possess features different from any other described species of *Claudopus*, namely abundant (10–75) basidiomata on the underside of granitic rocks and a pileus that is sticky to the touch. *Claudopus rupestris*, *C. viscosus,* and *C. minutoincanus* are described as new to science and are reported for the first time from Australia.

Materials & methods

Macromorphological and micromorphological features

Collections were made during February–April in 2009 and 2010 within the Wet Tropics Bioregion throughout northeastern Queensland and in April 2010 from various localities in central New South Wales. GPS coordinates for each collection were taken in the field using a Garmin GPSmap 60CSx. Basidiomata were carefully collected in the field, stored in plastic containers, and returned to the laboratory. Macroscopic descriptions were made from recently collected fresh material. Colors were described both subjectively and coded according to Kornerup & Wanscher (1978), with color plates noted in parentheses. Abbreviations of color plates used in specific descriptions indicate the page number, column(s), and row(s) [e.g., 8D-F5-6 indicates page 8, columns D–F, rows 5–6.]

Micromorphological features of dried specimens were examined with a trinocular research-grade Nikon Labophot compound microscope fitted with bright field light contrast optics following general protocols set forth in Largent (1994: 1–3) and techniques used for measuring spores of the *Entolomataceae* set forth by Baroni & Lodge (1998: 681). Digitized microphotographs were made using a Nikon Coolpix 990 focused through the trinocular head of the compound microscope. All microscopic measurements were obtained using a GTCO Corporation Graphic Digitizer, Model DP5A-111A connected to an IBM compatible Chem Book Laptop computer. The Measure Me 101 v 1.0 software used was modified for laptop use from a BASIC program (Metrics5) developed and provided by David Malloch, University of Toronto. Factors determined using this program include: arithmetic means (x_m) of basidiospore lengths

and widths ± standard deviation measured for n objects; quotient of basidiospore length by spore width (E) indicated as a range variation in n objects measured; the mean of E-values (Q) ± standard deviations. The sample size (n) = total number of microscopic structures measured (x) divided by the number of basidiomata studied (y), as shown in the formula n = x/y.

Molecular data

Dried basidiomata tissues were pulverized with glass beads in a FastPrep FP120 homogenizer (QBiogene, Carlsbad CA USA) at 4 mps for 20s. Genomic DNA was extracted using a 2 × cetyl trimethylammonium bromide (CTAB) modified method, and then purified on GeneClean Turbo columns (MP Biomedicals, Solon OH USA) following the Baumgartner et al. (2010). Three loci were PCR (Polymerase Chain Reaction) amplified including a portion of the mitochondrial small subunit ribosomal DNA (mtSSU) using primers MS1 and MS2 (White et al. 1990) following the PCR and cycling protocols of Gomes et al. (2000). The variable domains D1 and D2 of the 28S ribosomal DNA (LSU) were PCR amplified with the primers ctb6 and tw13 (White et al. 1990) following the PCR and cycling protocols outlined in Bergemann & Garbelotto (2006). The RNA polymerase II subunit II (RPB2) was amplified with primers rpb2i6f or rpb2i7r following protocols described in Co-David et al. (2009).

PCR products were cleaned using 1 µL of ExoSAP-IT (GE Healthcare, Pittsburgh, PA) and incubation of 37°C for 15 min followed by 80°C for 45 min. Sequencing reactions were carried out in 10 µL reactions containing 1 µL of ABI BigDye ver. 3.1 (Applied Biosystems, Carlsbad CA USA), 0.4 µM primer, 0.875 Sequencing Buffer (Applied Biosystems, Carlsbad CA USA) and 1 µL PCR water. Cycling conditions were as follows: 39 cycles of 96°C for 10 s, 50°C for 5 s, 60°C for 4 min. Reactions were precipitated with 1 µL of 3 M NaOAc, 1 µL 250 mM EDTA (pH 8) and 25 µL of 100% EtOH by centrifugation at 2500 G for 30 min. Reactions were rinsed with 70% EtOH and centrifuged at 2500 G for 15 min. Precipitated products were stored at -20°C. Bi-directional sequences were generated on an Applied Biosystems 3130xl Genetic Analyzer at Middle Tennessee State University. The contigs of bi-directional sequences were edited in Sequencher 4.8 (GeneCodes Corp., Ann Arbor, MI USA). Sequences for each new species from three loci were deposited in GenBank (HQ731511–731518), with the exception of the RPB2 sequence from DL Largent 9624 (*Claudopus rupestris*), omitted after repeated unsuccessful attempts to obtain sequences from weak PCR amplifications. The GenBank designators for each sequence of all three loci are provided at the end of each holotype description together with type habitat data and GPS co-ordinates. Actual sequences can be obtained from GenBank using the designator number. All of the sequences will be used in a later phylogenetic study.

Taxonomy

Claudopus rupestris Largent & Abell-Davis, **sp. nov.** Plate 1

MycoBank MB 519408

Ab aliis congeneris combinatione characterum sequentium differt: habitatio saxatilis; pileus lucens viscidusque; basidiosporae plerumque quadrangulae, interdum quinqangualae, subisodiametricae, 6.5–9.2 × 5.9–8.0 µm, longitudine mediale < 8.5 µm; basidia sterigmatibus 5–7; cystidia hymenialia cylindrica vel anguste obclavata; fibulae absens.

Type — Australia, Queensland, Cook Region, Wooroonooran National Park, Josephine Falls Track, within 50 m of 17°26′00.9″S, 145°51′33.8″E, 26 February 2009, DL Largent 9624 (mtSSU HQ731512, LSU HQ731515) (**holotype** BRI; **isotype** CNS).

Etymology — derived from Latin word *rupestris,* 'rock-dwelling'.

Basidiomata very abundant (50–75) and most often sessile to laterally stipitate. Pileus 1–4 mm broad, 1–3 mm deep, flabelliform to dimidiate, white with very pale yellowish tinges, minutely matted-fibrillose, glistening and sticky, even, opaque, not hygrophanous; margin decurved, even to irregularly lobed and not striate. Taste indistinct. Odor mild. Lamellae 5–8 per basidiome, 1–3 mm long, less than 1 mm high, white, adnexed, minutely ventricose, distant. Lamellulae 1 between lamellae; margin smooth and concolorous. Stipe at first centrally stipitate, then laterally stipitate, and then most often absent, 1 mm or less long, less than 0.75 mm broad, equal white and at first minutely pruinose then glabrous; stipe base or basidiomata attachment with minute hair-like rhizoids.

Basidiospores 4–5-angled in profile view, 5-angled in side view, angles distinct to indistinct but not rounded in any view, isodiametric to subisodiametric, rarely heterodiametric, on the average subisodiametric, 6.5–9.2 × 5.9–8.0 µm (x_m = 8.1 ± 0.62 × 6.8 ± 0.44 µm; E = 1.0–1.36; Q = 1.18 ± 0.10; n = 27/1). Basidia commonly 2 or 4-sterigmate, uncommonly 5–7-sterigmate, 20.8–31.8 × 8.0–11.5 µm (x_m = 27.19 ± 2.92 × 9.7 ± 1.02 µm; E = 2.45–3.10; Q = 2.81 ± 0.24; n = 15/1). Hymenial cystidia not readily visible on edge of entire gills, rare to scattered in squash mounts, cylindric, cylindro-clavate, or narrowly obclavate, 31.1–48.2 × 3.8–7.8 µm; n = 8/1. Pileipellis an entangled layer of inflated hyphae. Pileocystidia cylindro-clavate to broadly cylindro-clavate, 25.2–81.5 × 6.2–13.9 µm, (x_m = 42.0 ± 15.3 × 9.2 ± 2.0 µm; E = 2.7–5.4; Q = 4.55 ± 1.32; n = 13/1). Pileal trama 26.5–41.6 µm deep. Tramal hyphae in pileus, lamellae and stipe not measured because of minute size of basidiomata. Stipitipellis not examined. Oleiferous hyphae absent. Lipoid bodies absent. Clamp connections absent in all tissues.

Habit, habitat and distribution gregarious on undersurface of small (25 × 25 cm) granitic rocks growing on 1 mm thin to nearly non-existent soil layer with minute mosses; basidiomata apparently attached to the rhizoids of the moss gametophyte; complex mesophyll forest. Known only from the type locality.

Diagnostic characters: basidiomata 1–4 mm diameter, growing on soil with minute mosses on the undersurface of small granitic rocks; basidiospores in profile view commonly 4-angled, sometimes 5-angled, on the average subisodiametric (6.5–9.2 × 5.9–8.0 µm; x_m = < 8.5 µm; E = 1.18). Basidia small, occasionally 5–7-sterigmata, hymenial cystidia rare to scattered, cylindric to cylindro-clavate to narrowly obclavate, clamps absent.

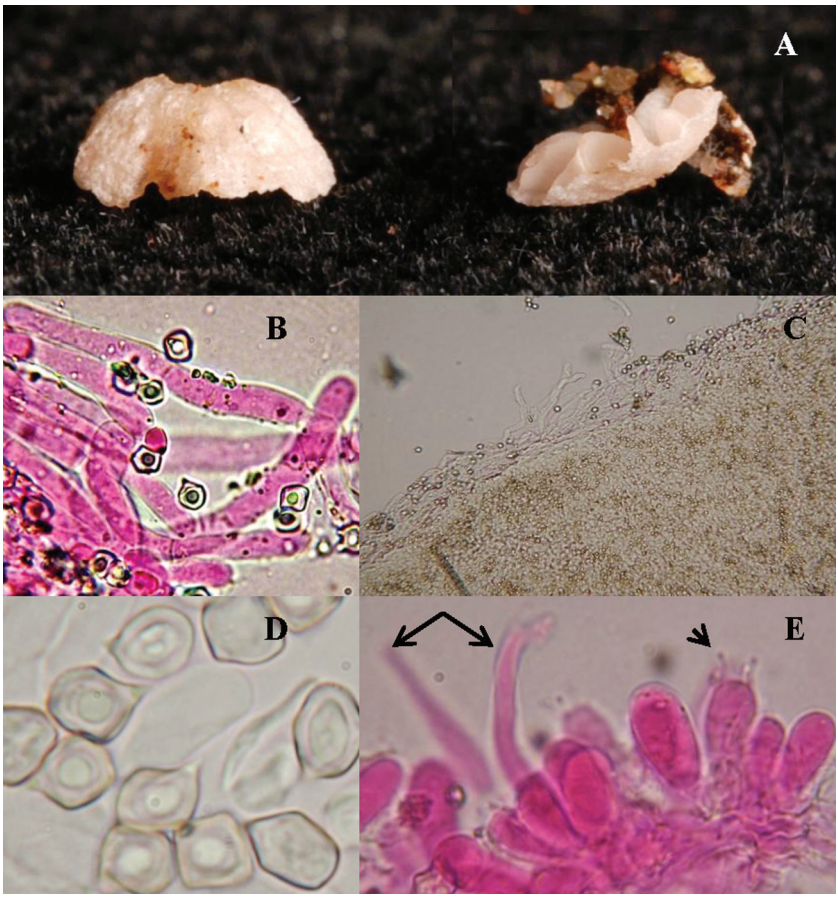

Plate 1 – *Claudopus rupestris* (DL Largent 9624: Holotype). A: Basidiomata (9×); B: Pileocystidia (400×); C: Pileipellis (100×); D: Basidiospores (1000×); E: Cheilocystidia and Basidia (400×).

Comments— In Australia, *Claudopus rupestris* is macroscopically similar to *C. viscosus* and *C. minutoincanus*, both also found in saxicolous habitats. The 4–5-angled basidiospores and absence of subcapitulate to capitulate pileocystidia distinguish the *C. rupestris* from *C. viscosus* and *C. minutoincanus,* both of which lack 4-angled basidiospores and have subcapitulate to capitulate pileocystidia.

Taxa related to *Claudopus rupestris*

Entoloma albotomentosum Noordel. & Hauskn. (from central Europe and Norway) and *Claudopus pandanicola* E. Horak (from Papua New Guinea) morphologically resemble *C. rupestris* in their pleurotoid, white basidiomata

with a fibrillose pileus, mild taste or odor, occasionally 4-angled or similar sized basidiospores, and absence of clamp connections. *Entoloma albotomentosum* can be differentiated by its longer (6 mm) stipe, non-sticky, larger pileus (2–10 mm broad), adnate or decurrent, very distant lamellae, translucent-striate pileus when expanded, longer (9–12.5 µm) 4–6-angled basidiospores, and habitat on leaves of grasses and sedges in marshes (Noordeloos 1992). *Claudopus pandanicola* can be distinguished by the larger (5–15 mm broad) non-sticky pileus, habitat on rotting *Pandanus* leaves, 5–6-angled basidiospores (none of which are rectangular), and pigment-encrusted pileipellis hyphae (Horak 1980).

Claudopus viscosus Largent & Abell-Davis, **sp. nov.** PLATE 2

MYCOBANK MB 519409

Ab Entolomate *(subg.* Claudopo*) jahnii habitatione saxatili, pileo viscido margine striato minute sulcato, sapore odoreque subtiliter farinaceo, basidiosporis 7.7–12.0 × 5.3–7.9 µm (mediane 9.8 × 6.7 µm), fibulis absentibus differt.*

TYPE — Australia, Queensland, Cook Region, Danbulla National Park, Kauri Creek Track, 17°07′56.3″S, 145°35′54.3″E, 17 March 2010, DL Largent 9788 (mtSSU HQ731513, LSU HQ731516, RPB2 HQ731518) (**holotype** BRI; **isotype** CNS).

ETYMOLOGY — derived from the Latin word *viscosus,* referring to the 'sticky' surface of the basidiomata.

BASIDIOMATA shiny and sticky to fingers, forceps, knives, leaves and debris; attached by minute rhizomorphs to rhizoids of moss gametophytes. PILEUS 1.5–11.0 mm broad, 2–10 mm deep, 1–1.5 mm high, broadly convex from side view, not hygrophanous, at first white and opaque, entirely matted-fibrillose and minutely petaloid to flabelliform in top view with an incurved to decurved margin, upon expansion and maturity becoming more flabelliform and ± dimidiate with a decurved, plane, or uplifted margin that is eroded and crenulate and in some of the broader specimens the fibrillose layer disappears with age revealing a ± glabrous undersurface that is minutely sulcate, suggestively striate and off white to pinkish-white; striate in dried specimens. TASTE and ODOR latently farinaceous. LAMELLAE up to 6 mm long and 2.5 mm high, adnexed at the point of attachment, white at first, moderately broad and close, quickly becoming broad to ventricose and subdistant to distant; margin even and concolorous. LAMELLULAE 1–3 (1–2 short, 1 medium long) between lamellae. STIPE 1.0 × 0.5 mm and covered with minute, white hairs in very young specimens, with maturity and expansion of the pileus, the hyphae of the stipe quickly merge with the hyphae of the pileus or pileus surface and thus is absent in older specimens.

BASIDIOSPORES 5–6-angled, subisodiametric to more typically heterodiametric in profile and side views, 6-angled and isodiametric in polar view, 7.7–12.0 × 5.3–7.9 µm (x_m = 9.8 ± 0.76 × 6.7 ± 0.57 µm; E = 1.22–1.88;

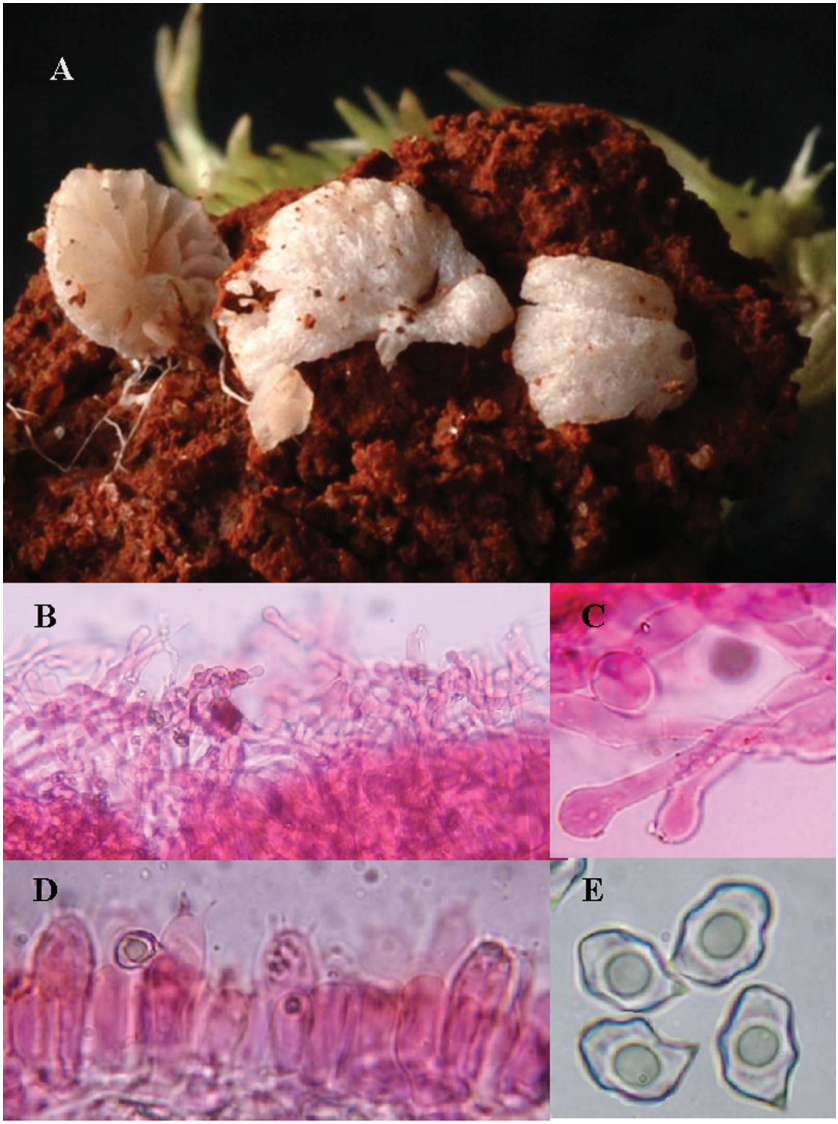

Plate 2 – *Claudopus viscosus* (DL Largent 9788: Holotype). A: Basidiomata (4×); B: Pileipellis at point of attachment (100×); C: Capitulate pileocystidia (400×); D: Basidia and basidioles (400×); E: Basidiospores, all profile view (1000×).

Q = 1.47 ± 0.13 (heterodiametric); n = 16/4). Basidia small, clavate to cylindro-clavate, 23.6 × 33.6 × 8.0–11.5 µm (x_m = 29.5 ± 2.64 × 10.0 ± 0.84 µm; E = 2.48–3.79; Q = 2.97 ± 0.30; n = 16/2); 1-, 2-, 3- or 4-sterigmate. Hymenial

cystidia absent. Hyphae of lamellar trama thin-walled, subparallel, relatively short, 90.0–122.6 × 15.7–18.7 µm. Pileipellis an entangled layer of hyphae, 80–224 µm deep. Pileocystidia with a granular surface, frequently subcapitulate to capitulate, 35.66–79.64 × 2.31–8.15 µm, capitulum 4.5–10.9 µm broad. Oleiferous hyphae absent. Lipoid bodies absent. Pigmentation non-existent. Clamp connections absent in all tissues.

Habit, habitat and distribution scattered to gregarious in a thin layer of soil in amongst and attached to the rhizoids of few, tiny moss gametophytes on the underside of granitic rocks in simple to complex notophyll vine forest, northeastern Queensland.

Additional collections examined — **AUSTRALIA**, **Queensland**, Cook Region, Dinden National Park, near end of Davies Creek Road, 17°02′13.1″S, 145°36′47.8″E, 23 February 2010, DL Largent 9733; Danbulla National Park, Kauri Creek Track, 17°07′56.3″S, 145°35′54.3″E 9840 (three **topotypes**) – 17 March 2010, DL Largent 9789; 24 Mar 2010, DL Largent 9828; 27 Mar 2010, DL Largent. (All collections BRI; split collections CNS.)

Diagnostic characters: Basidiomata shiny and sticky to fingers, forceps, knives, leaves and debris; attached by minute rhizomorphs to rhizoids of moss gametophytes on the underside of granitic rocks. Pileus at first white, opaque, and entirely matted fibrillose then, in some, ± glabrous towards the margin and minutely sulcate and suggestively striate. Taste and odor latently farinaceous. Basidiospores 5–6-angled, measuring 7.7–12.0 × 5.3–7.9 µm (average = 9.8 × 6.7 µm). Cheilocystidia and clamp connections are absent.

Comments— In Australia, *C. viscosus* and *C. minutoincanus* produce pleurotoid, sticky basidiomata with a white fibrillose pileus and subcapitulate to capitulate pileocystidia, and both are found in a saxicolous habitat. The mild taste and odor, sulcate striate mature pileus lacking yellowish tinges, smaller basidia, and longer narrower heterodiametric (E = 1.47) basidiospores distinguish *C. viscosus* from *C. minutoincanus* with its latently farinaceous taste and odor, yellow-tinged opaque even mature pileus, longer larger basidia (33–38 × 10–13 µm), and shorter and broader subisodiametric basidiospores (E = 1.22) averaging 9.1 × 7.4 µm. *Claudopus rupestris* (Wooroonooran National Park, Queensland) is also saxicolous but lacks subcapitulate to capitulate pileocystidia and has 4–5-angled basidiospores.

Taxa related to *Claudopus viscosus*

Entoloma jahnii Wölfel & Winterh. (from Germany and Belgium), which produces pleurotoid basidiomata with white fibrillose pilei and capitulate pileocystidia, is differentiated from *C. viscosus* and *C. minutoincanus* by its mild taste and odor, larger basidiospores (9.7–12.9(–15) × 7.6–11(–15) µm), presence of clamp connections, and habit on rotting wood and bark (Noordeloos 2004).

Claudopus minutoincanus Largent & Abell-Davis, **sp. nov.** PLATE 3

MYCOBANK MB 519410

Ab Entolomate *(subg.* Claudopo*) jahnii habitatione saxatili, pileo viscido luteoalbo suffuso, basidiosporis 7.4–11.4 × 6.3–9.6 µm (mediane 9.01 × 7.4 µm), fibulis absentibus differt.*

TYPE — Australia, New South Wales, Central Hunter District, Barrington Tops National Park, Williams Day Use Area, end of Blue Gum Loop, 32°09′11.0″S, 151°31′38.9″E, 14 April 2010, DL Largent 9871 (mtSSU HQ731511, LSU HQ731514, RPB2 HQ731517) (**holotype** DAR).

ETYMOLOGY — derived from a combination of the Latin *minutus* + *incanus,* referring to the 'minutely' 'hoary' pileal surface.

BASIDIOMATA attached by minute stipe as well as the basal portion of pileus. PILEUS in side view convex, 1–3 mm high, in top view minutely petaloid then dimidiate, 6 mm broad, 2–5 mm deep, whitish with a hint of yellowish-white (3A2), matted fibrillose 2/3 way to margin, minutely tomentulose-canescent towards the margin and with an incurved then decurved and eventually uplifted, minutely canescent margin, glistening and sticky, dull, even, opaque, not hygrophanous. TASTE and ODOR indistinct. LAMELLAE adnexed, 3 mm long, 0.5 mm high, narrow, close, yellowish-white (3A2); margin smooth and concolorous. STIPE lateral and minute < 1 mm long, < 0.5 mm broad, white, covered with fine hairs that disappear when dried, equal; basal tomentum absent.

BASIDIOSPORES 6-angled, isodiametric to heterodiametric in profile and side views, 6-angled and isodiametric in polar view, 7.4–11.4 × 6.3–9.6 µm (x_m = 9.01 ± 0.94 × 7.4 ± 0.77 µm; E = 1.08–1.44; Q = 1.22 (subisodiametric) ± 0.09; n = 30/1). BASIDIA clavate, medium in length, 4-sterigmate, 32.7–38.3 × 9.8–13.2 µm (x_m = 34.9 × 11.5 µm; E = 2.75–3.41l; Q = 3.05; n = 8/1). HYMENIAL CYSTIDIA absent. PILEIPELLIS a superficial entangled layer of hyphae, scant at the point of attachment to the substrate and then transitioning into an erect palisadoderm of slender pileocystidia. PILEOCYSTIDIA cylindric and subcapitulate to capitulate, suggestively covered with a thin layer of material, 14.4–68.3 µm long, 2–3 µm broad, capitulum 3.6–6.3 µm near point of attachment, at the margin particularly abundant, 39.5–53.1 µm long, 2–3 µm broad, capitulum 4.1–6.4 µm. STIPITIPELLIS not examined. OLEIFEROUS HYPHAE absent. LIPOID BODIES absent. PIGMENTATION non-existent. CLAMP CONNECTIONS absent in all tissues.

HABIT, HABITAT AND DISTRIBUTION scattered on thin layer of soil and mosses beneath a rock, subtropical gallery rainforest. Known only from type locality.

DIAGNOSTIC CHARACTERS Basidiomata glistening, sticky, and laterally stipitate; pileus white with a hint of yellowish-white, matted fibrillose, minutely canescent near or at the margin, taste and odor mild; basidiospores 6-angled,

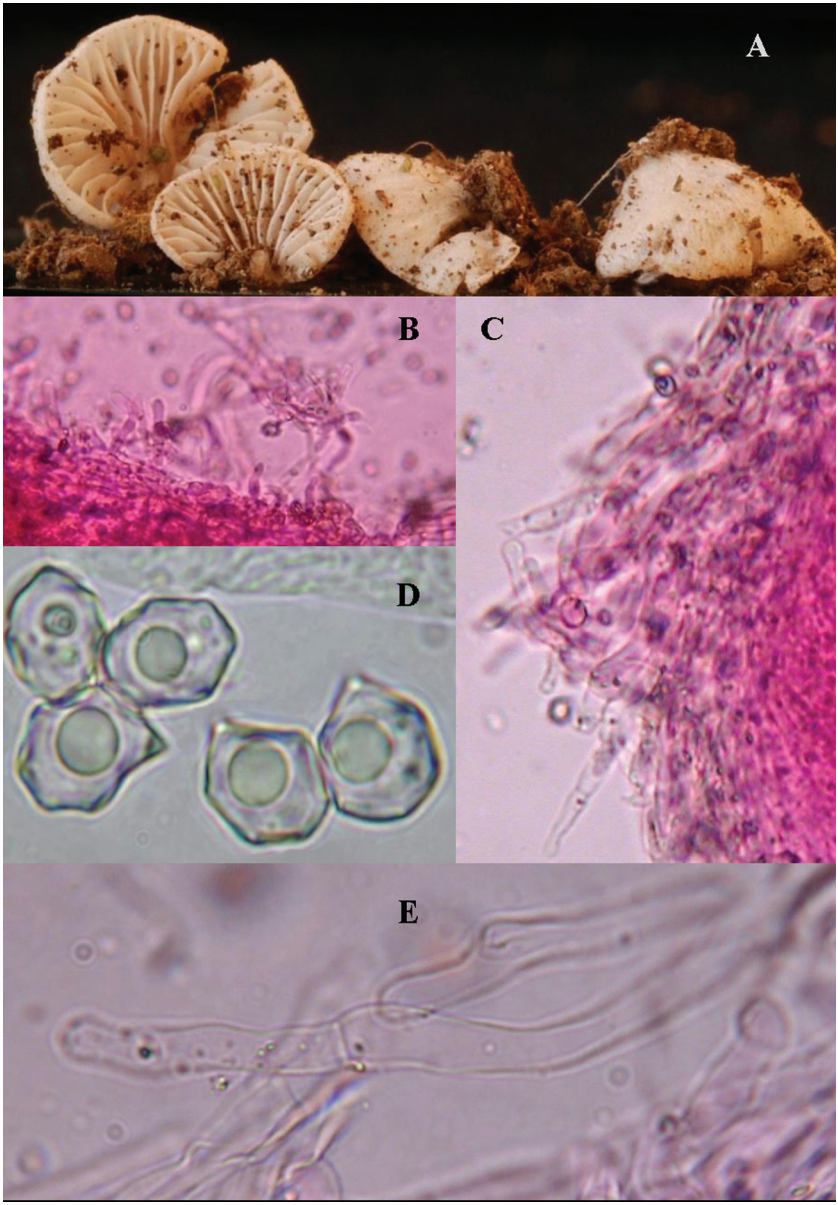

Plate 3 – *Claudopus minutoincanus* (DL Largent 9871: Holotype). A: Basidiomata (7×); B: Pileipellis near point of attachment (120×); C: Pileipellis at pileus margin (250×); D: Basidiospores (1000×); E: Subcapitulate to capitulate pileocystidium left (400×).

on the average subisodiametric (E = 1.22), and with an average length ≤ 9.01 µm, subcapitulate to capitulate pileocystidia; lack of hymenial cystidia and clamp connections.

Comments— Please refer to the comments under *Claudopus viscosus*.

Discussion

All the basidiomata collected for the species described in this report stuck to the surface of the collecting implements as well as fingers, the surface of the collecting boxes, leaves, and pieces of paper. The surface hyphae of the basidiomata glistened as if covered by a thin gelatinous material. A very thin layer of colorless, non-staining, amorphous material was observed on the capitulate to subcapitulate pileocystidia of *Claudopus minutoincanus* and *C. viscosus*. No discernable material could be observed on any other hyphae of the pileipellis of these two species and on any hyphae of any part of the basidiomata of *C. rupestris*. There currently is no viable explanation for the stickiness of the basidiomata.

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